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NLR immune receptors and diverse types of non-NLR proteins control race-specific resistance in *Triticeae*

Javier Sánchez-Martín and Beat Keller

Abstract

Recent progress in large-scale sequencing, genomics, and rapid gene isolation techniques has accelerated the identification of race-specific resistance (*R*) genes and their corresponding avirulence (*Avr*) genes in wheat, barley, rye, and their wild relatives. Here, we describe the growing repertoire of identified *R* and *Avr* genes with special emphasis on novel *R* gene architectures, revealing that there is a large diversity of proteins encoded by race-specific resistance genes that extends beyond the canonical nucleotide-binding domain leucine-rich repeat proteins. Immune receptors with unique domain architectures controlling race-specific resistance possibly reveal novel aspects on the biology of host–pathogen interactions. We conclude that the polyploid cereal genomes have a large evolutionary potential to generate diverse types of resistance genes.

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Keywords

R gene, Race-specific resistance, NLR, Non-NLR proteins, Effector-triggered immunity.

Introduction

Genetic analysis of plant disease resistance against adapted pathogens has revealed two distinct, main forms of resistance in response to pathogen infection. Race-specific resistance provides mostly complete resistance to some races of a pathogen species and is controlled by single resistance (*R*) genes. Resistance only occurs in the presence of an *R* gene and the

corresponding pathogen avirulence (*Avr*) gene [1]. In contrast, non–race-specific resistance provides mostly partial, quantitative resistance (QR) to all races of a pathogen species and is independent of specific avirulence genes. QR delays disease development, and several quantitative trait loci act additively to confer resistance [2]. Some of the genes underlying these quantitative trait loci may make major contributions to QR. This is the case for the wheat genes *Lr67/Yr46* [3] and *Lr34/Yr18/Pm38* [4] that provide partial, QR against leaf rust, stripe rust, stem rust, and powdery mildew or *Fhb7* against *Fusarium* [5]. In addition to race-specific and QR genes, there are susceptibility genes, like the barley *Mlo* gene, where loss-of-function was found to confer recessively inherited resistance to virtually all the barley powdery mildew isolates [6].

Different types of plant immune receptors recognize pathogen-derived molecules initiating differential defense responses which then converge into common signaling pathways [7]. Pattern recognition receptors, PRRs, (either receptor-like kinases or receptor-like proteins) recognize conserved pathogen-associated (or microbial-associated) molecular patterns, triggering pattern-triggered immunity (PTI) to induce defense reactions against nonadapted pathogens. Residual levels of PTI were proposed to provide basal resistance against adapted pathogens [8,9], which would place PTI within non–race-specific resistance. *Triticeae* PRRs remain largely unidentified, although some candidates have been pinpointed [10]. Most race-specific *R* genes identified encode nucleotide-binding domain leucine-rich repeat (NLR) immune receptors that recognize pathogen strain–specific effectors. The resulting effector-triggered immunity efficiently stops pathogen spread.

Hundreds of *R* genes have been genetically described in the major cereal crops [11] and remain as an essential pillar for disease resistance breeding. Recent gold standard genomic resources [12–15] alongside innovative gene cloning strategies have greatly facilitated *R* gene cloning [16], providing tangible opportunities to broaden disease resistance diversity in crops. Here, we focus on recent progress in the molecular identification of race-specific resistance genes in wheat, barley, rye, and wild relatives. These genes include the canonical *R* genes encoding NLR proteins, but also an increasing number of novel immune receptors with unique domain

Box 1.**The genetic definition of a resistance gene**

Resistance genes have been identified by phenotypic and genetic studies for more than hundred years, long before gene cloning was possible. They are widely used in crop breeding programs and remain as important sources of disease resistance. In wheat, several hundred genes are known for resistance to the three rust diseases leaf, stem, and stripe rust as well as powdery mildew. The term 'resistance gene' has been used to describe the polymorphic genetic component that makes the crucial difference between resistant and susceptible genotypes within a species. At the molecular level, the product of a resistance gene frequently recognizes (directly or indirectly) the presence of the pathogen, but this must not necessarily be the case. For example, a nonpolymorphic gene acting upstream of the resistance gene might detect a pathogen molecule, and a downstream component required for signal transduction and defense response induction could be the component whose presence or absence would determine if a genotype is resistant. This downstream component would be then called the resistance gene. It would be polymorphic in the gene pool of the crop species, whereas the upstream component sensing the pathogen, e.g. an NLR protein, would be nonpolymorphic. Therefore, not all *R* genes necessarily encode typical immune receptors such as NLRs or receptor-like proteins, and race specificity does not depend on a specific protein structure such as NLR. It is mostly unknown how the non-NLR proteins described in this review provide race-specific resistance, i.e. if they are immune receptors, are downstream components in a signaling cascade, or have other molecular functions. It is expected that the study of their functions will reveal novel biological aspects of plant disease resistance.

architectures, possibly revealing novel aspects of host–pathogen interactions. Importantly, the term resistance gene is not associated with a specific molecular characteristic, but rather based on a phenotypic and genetic characterization (Box 1).

NLR-based race-specific resistance in *Triticeae* is highly diversified and polymorphic

A growing number of wheat, barley, and rye resistance genes have been molecularly isolated [16,17], including genes from wild relatives that are functional in wheat (Table 1). They are mostly active against fungal diseases but can also confer resistance to aphids [18]. Some rye genes have been cloned from rye chromosomal translocations introgressed into wheat [19–21]. This has also revealed that members of the same NLR gene cluster have evolved into stem rust resistance genes in wheat (*Sr33*) and rye (*Sr50*) or powdery mildew resistance genes in barley and wheat (*Mla* allelic series), raising interesting evolutionary implications on conserved effector function and recognition [22]. For most of the isolated genes, forward genetic screens revealed only one complementation group. This suggests that additional genes are not necessary for race-specific resistance. Alternatively, these genes might be redundant or

essential, thereby escaping detection by mutagenesis. Based on the results from the molecular identification of 40 *R* genes, we estimate that around 85% of wheat race-specific resistance genes encode NLRs. However, this might be an overestimation as molecular identification has focused on genes that are used in breeding, excluding genes in older landraces or nondomesticated wheat, as well as genes with narrower specificity potentially less useful for breeding. To study the molecular interactions of NLRs with pathogen molecules, with interaction specificity sometimes depending on a single amino acid [23], several avirulence genes have been recently isolated (Table 1). However, the identification of avirulence genes lags behind resistance genes.

Recent genomic analysis has revealed the complete sets of NLR coding genes in wheat. In the reference genome of cultivar Chinese Spring, 3400 full-length NLR loci were detected, with 1560 of them expressed and with intact open reading frames [24]. The pan-‘NLRome’ of wheat, that is the complete diversity of NLR coding genes in the gene pool, was estimated by comparing 10 high-quality genomes from diverse wheat elite varieties. Among the ten genomes studied, only 31–34% of the NLR signatures were found across all genomes, whereas the number of unique NLR signatures ranged from 22 to 192. Furthermore, it was estimated that 10 wheat genomes reveal about 90% of all NLR genes present in the wheat gene pool, which is estimated to consist of 5905 to 7780 unique NLR genes [25]. Thus, the theoretical maximal number of NLR-based, race-specific resistance genes (excluding allelic variants) in wheat is around 7,000, of which less than 10% would have been genetically described until now. However, the wheat pan-‘NLRome’ could be even larger. The lines selected in the 10+ Wheat Genomes Project cover well the global genetic diversity of elite wheat cultivars, but they possibly do not represent the complete diversity present in landraces and in the diploid and tetraploid genomes of wheat.

Some NLR genes have unique functional aspects only partially understood. For example, both expression as well as resistance phenotype increased with temperature for the *Sr21* gene [26]. Moreover, *Sr21* expression and resistance were lower in wheat genotypes with a D genome. D genome-based suppression was also observed for stem rust resistance in the cultivar ‘Canthatch’. However, it is not known if the suppressed stem rust resistance gene encodes an NLR. The suppressor on the D genome of ‘Canthatch’ was identified as a subunit of the mediator complex which is conserved in eukaryotes and regulates gene expression [27]. Suppression of the rye NLR Pm8 by an ortholog in wheat was found to occur at the protein level, a mechanism which might be responsible for the frequent suppression of genes introgressed into bread wheat from diploid or tetraploid wheat relatives [28].

Table 1

List of cloned race-specific resistance genes against fungal pathogens isolated in wheat, barley, rye, and wild relatives and their corresponding cloned Avr genes.

Donor species ^a	Gene	Gene product	Disease, causal agent	Immune trigger
<i>Triticum aestivum</i>	<i>Lr1</i> [68]	NLR	Wheat leaf rust, <i>Pt</i>	–
<i>Triticum aestivum</i>	<i>Lr10</i> [69]	NLR		–
<i>Triticum aestivum</i>	<i>Lr14a</i> ^h [60]	ANK ^b		–
<i>Aegilops tauschii</i>	<i>Lr21</i> [70]	NLR		–
<i>Aegilops tauschii</i>	<i>Lr22a</i> ^h [71]	NLR		–
<i>Hordeum vulgare</i>	<i>Rph1</i> ^h [72]	NLR	Barley leaf rust, <i>Ph</i>	–
<i>H. vulgare</i> subsp. <i>spontaneum</i>	<i>Rph15</i> [73]	BED–NLR ^c		–
<i>Ae. tauschii</i> ssp. <i>strangulata</i>	<i>SrTA1662</i> ^h [74]	NLR	Wheat stem rust, <i>Pgt</i>	–
<i>Triticum turgidum</i> ssp. <i>durum</i>	<i>Sr13</i> [75]	NLR		–
<i>Triticum monococcum</i>	<i>Sr21</i> [26]	NLR		–
<i>Triticum monococcum</i>	<i>Sr22</i> ^a [76]	NLR		–
<i>Aegilops tauschii</i>	<i>Sr33</i> [77]	NLR		–
<i>Triticum monococcum</i>	<i>Sr35</i> [78]	NLR		<i>AvrSr35</i> [63]
<i>Aegilops tauschii</i>	<i>Sr45</i> ^h [76]	NLR		–
<i>Ae. tauschii</i> ssp. <i>strangulata</i>	<i>Sr46</i> ^h [74]	NLR		–
<i>Secale cereale</i>	<i>Sr50</i> [20]	NLR		<i>AvrSr50</i> [62]
<i>Triticum monococcum</i>	<i>Sr60</i> [44]	TKP ^d		–
<i>Hordeum vulgare</i>	<i>Rpg1</i> [35]	TKP ^d	Barley stem rust, <i>Pgt</i>	–
<i>Triticum aestivum</i>	<i>Yr5</i> , <i>YrSP</i> ^h [32]	BED–NLR ^c	Wheat yellow rust, <i>Pst</i>	–
<i>Triticum aestivum</i>	<i>Yr7</i> ^h [32]	BED–NLR ^c		–
<i>Triticum aestivum</i>	<i>Yr10</i> [79]	NLR		–
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i>	<i>Yr15</i> [36]	TKP ^d		–
<i>Aegilops tauschii</i>	<i>YrAS2388R</i> [80]	NLR		–
<i>Triticum urartu</i>	<i>YrU1</i> [33]	ANK–NLR–WRKY ^e		–
<i>Triticum aestivum</i>	<i>Pm1a</i> ^h [81]	NLR	Wheat powdery mildew, <i>Bgt</i>	<i>AvrPm1a</i> [81]
<i>Aegilops tauschii</i>	<i>Pm2</i> ^h [59]	NLR		<i>AvrPm2</i> [66]
<i>Triticum aestivum</i>	<i>Pm3b</i> [82]	NLR		<i>AvrPm3b</i> [65]
<i>Triticum aestivum</i>	<i>Pm3a,d</i> [83]	NLR		<i>AvrPm3a,d</i> [64,65]
<i>Triticum carthlicum</i>	<i>Pm4b</i> ^h [61]	MCTP kinase ^f		–
<i>Triticum aestivum</i>	<i>Pm5e</i> [23]	NLR		–
<i>Secale cereale</i>	<i>Pm8</i> [19]	NLR		–
<i>Secale cereale</i>	<i>Pm17</i> [21]	NLR		–
<i>Dasypyrum villosum</i>	<i>Pm21</i> ^h [84,85]	NLR		–
<i>Triticum aestivum</i>	<i>Pm24</i> [37]	TKP		–
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i>	<i>Pm41</i> [86]	NLR		–
<i>Triticum urartu</i>	<i>Pm60</i>	NLR		–
<i>Hordeum vulgare</i>	<i>Mla1</i> , 13 [87]	NLR	Barley powdery mildew, <i>Bgh</i>	<i>Avra1</i> , 13 [88]
<i>Hordeum vulgare</i>	<i>Mla7,9,10,22</i> and alleles [87]	NLR		<i>Avr7,9,10,22</i> [89]
<i>Triticum aestivum</i>	<i>Stb6</i> [50]	WAK	Septoria <i>tritici</i> blotch, <i>Zt</i>	<i>AvrStb6</i> [51,52]
<i>Triticum aestivum</i>	<i>Snn1</i> [55]	WAK	SNB, <i>Sn</i>	<i>SnTox1</i> [90]
<i>Triticum aestivum</i>	<i>Tsn1</i> [91]	K-NLR ^g	SNB, <i>Sn</i> ; tan spot, <i>Ptr</i>	<i>SnToxA</i> [56]
<i>Hordeum vulgare</i>	<i>rcs5</i> [58]	WAK	Barley spot blotch, <i>Bp</i>	–

Pt: *Puccinia triticina*; Ph: *Puccinia hordei*; Pgt: *Puccinia graminis* f. sp. *tritici*; Pst: *Puccinia striiformis* f. sp. *tritici*; Bgt: *Blumeria graminis* f. sp. *tritici*; Bgh: *Blumeria graminis* f. sp. *hordei*; Zt: *Zymoseptoria tritici*; Sn: *Stagonospora nodorum*, causal agent of SNB (*Stagonospora nodorum* blotch); Ptr: *Pyrenophora tritici-repentis*; Bp: *Bipolaris sorokiniana*.

^a Donor species refers to the original source of the resistance gene.

^b ANK: ankyrin-transmembrane domain protein.

^c BED–NLR: zinc-finger BED–NLR proteins.

^d TKP: tandem kinase proteins.

^e ANK–NLR–WRKY: ankyrin repeat and WRKY domain-containing NLR protein.

^f MCTP kinase: multiple C2-domains and transmembrane region kinase protein.

^g K-NLR: serine/threonine kinase–NLR; NLR: nucleotide-binding domain leucine-rich repeat (NLR) proteins; WAK: wall-associated receptor (WAK)-like protein.

^h Resistance genes for which molecular identification was carried out based on forward genetic screens, and only one complementation group was found. Barley *Rph1* is the exception with additional complementation groups found.

NLR resistance genes with integrated domains (IDs)

In addition to the domains found in typical NLR proteins, some wheat and barley NLRs contain IDs that may be involved in receptor activation or downstream signaling [29]. Some of these chimeric genes are ancient, and they originated before the speciation of grass lineages. For example, rice contains NLRs with zinc-finger BED domains [30,31]. In wheat, two active resistance genes encoding ID-NLRs have recently been cloned. First, the wheat stripe rust resistance genes *Yr5*, *Yr7*, and *YrSP* encode proteins with an N-terminal noncanonical zinc-finger BED domain [32]. The BED domain replaces the coiled coil domain present in canonical NLR proteins, and it is followed by the NB-ARC and the LRR domains. Mutant analysis shows that this BED domain is critical for resistance and displays a high degree of sequence conservation among the BED–NLR proteins encoded by the *Yr5*, *Yr7*, and *YrSP* genes, implying the BED domain plays a major role in protein function. Moreover, each gene has a distinct recognition specificity, attributable to the numerous polymorphisms in the C-terminal LRR domain. Therefore, it is assumed that race-specificity in BED–NLR proteins is controlled similarly to canonical NLR proteins (Figure 1).

The wheat *YrU1* gene encodes an ID-NLR with ID domains at both its C and N termini: an N-terminal ankyrin repeat and a C-terminal WRKY domain [33]. This type of ID-NLR protein is only found in wheat-related species, and it self-associates *in vivo* and in planta through the CC and ANK domains. The WRKY domain is a putative transcriptional domain that might be involved in recognition of a stripe rust effector to activate immune response similarly to the Arabidopsis resistance protein complex RPS4/RRS1 [34] (Figure 1).

Tandem kinase proteins (TKPs) can confer both race-specific as well as non–race-specific resistance

Four *Triticaceae* resistance genes, the barley stem rust resistance gene *Rpg1* [35], the wheat yellow and stem rust resistance genes *Yr15* [36] and *Sr60* [37], and the wheat powdery mildew resistance gene *Pm24* [37], encode resistance proteins with a protein architecture made of two tandem kinase (or pseudokinase) domains. All these TKPs belong to serine/threonine non-RD (non-arginine-aspartate) kinases, previously shown to be involved in plant immunity [38], and have evolved by either fusion or duplication of two kinase domains. Based on the presence of conserved residues of serine/threonine protein kinases [39] (Figure 2), at least one of the two kinase domains is functional. Besides, both kinase and pseudokinase domains are required to confer resistance in mutant and transgenic analysis [35–37].

TKP-mediated resistance is associated with hypersensitive response [35–37], indicative of an effector-triggered

immunity-like resistance response. Moreover, *Yr15* and *Rpg1* are cytosolic proteins [36,40] (Figure 1), suggesting recognition of intracellular effectors. However, resistance spectra exhibited by these genes differ. Although *Sr60* confers resistance to some of the (few) wheat stem rust races tested, *Yr15* was shown to protect against dozens of genetically and geographically diverse yellow rust races [36] and *Pm24* to 93 Chinese *Bgt* isolates. Moreover, *Rpg1* has provided remarkable durable resistance under field conditions spanning decades [41]. Consequently, *Yr15* and *Rpg1* have been considered as non–race-specific resistance genes. However, as (few) virulent races breaking *Rpg1*- and *Yr15*-mediated resistance have been identified [42,43], TKP-encoding genes must possibly be considered as race-specific. Few polymorphic alleles among TKP-encoding genes have been reported [37,44], which contrasts to canonical NLR-encoding *R* genes where multiple functional allelic series and rapid diversification of resistance clusters have been described.

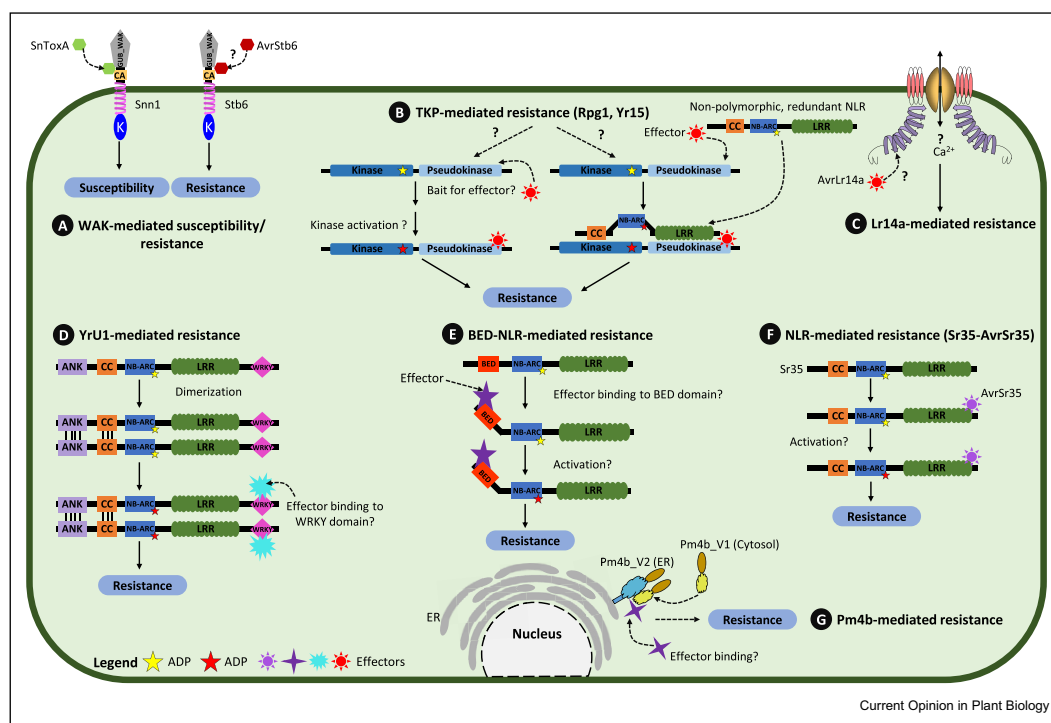
Interestingly, *Yr15* introgression lines display different resistance phenotypes [36,45], indicating that the genetic background is important for gene function, and additional genetic components play a role in TKP-mediated resistance. A similar situation has been observed in *Pto*-mediated resistance among wild tomato populations, displaying resistance variation of *Pto* alleles recognizing the corresponding *AvrPto* genes [46] (Figure 1). Here, it is believed that malfunction of some of *Pto* response network genes would result in such resistance variation.

TKP proteins contain a kinase domain with serine/threonine specificity with strong homology to *Pto* and PRRs (Figure 2). *Pto* functions together with the NLR protein Prf to confer resistance against bacterial pathogens in tomatoes [47]. It could be that the diverse, genetic background-dependent resistance responses by *Pto* result from the presence/absence of as yet unknown genetic components also involved in TKP-mediated resistance. In *Rpg1*-mediated resistance, E3 ubiquitin ligase SCF (Skp1-cullin 1-F-box) complex components seem to be involved in resistance function [48]. It is likely that additional genetic components modulate TKP-mediated resistance. Finally, it has been hypothesized that the pseudokinase domain serves as decoy for the effectors, and after interaction, the pseudokinase activates the kinase domain to phosphorylate downstream components resulting in resistance [36] (Figure 2). Evidently, the elucidation of TKP-mediated signaling will require additional work to establish the molecular mechanism underlying this resistance.

Wall-associated kinases (WAKs)

WAKs are receptor-like kinases with an extracellular galacturonan-binding domain, transmembrane domain, and cytoplasmic serine threonine kinase domain with a

Figure 1



Schematic representation of a plant cell and models for race-specific resistance gene function. (A) **WAK-mediated susceptibility/resistance.** SnToxA secreted by the pathogen is recognized in the apoplast by direct interaction with the Snn1 WAK protein. On recognition, signaling leads to the induction of cell death and *Septoria nodorum* blotch disease. On the other side, the Stb6 WAK protein detects the corresponding apoplastic AvrStb6, leading to a strong resistance response and completely blocking *Septoria tritici* blotch disease progression. (B) **TKP-mediated resistance (Rpg1, Yr15).** The signaling cascade for these cytosolic proteins is currently unknown. It has been proposed that pseudokinase domains might serve as decoys for rust effectors. In this case, on effector recognition, the pseudokinase protein may act as a 'molecular switch' to activate the kinase domain. Alternatively, based on the high similarity at the sequence level between the kinase domains of TKP proteins and PRRs (Figure 1), TKPs might be guarders of nonpolymorphic, possibly redundant NLRs. The pseudokinase domain would act as a cofactor (bait) to stabilize the interaction with the NLR, which results in inhibition of the latter. The binding of the effector to the pseudokinase would trigger a structural change in the TKP protein and subsequently activate the NLR. (C) **Lr14a-mediated resistance.** The *Lr14a* gene encodes a membrane-bound protein with multiple ankyrin domains and structural similarities to nonselective cation channels. It remains unclear if Ca^{2+} plays a role in Lr14a-mediated resistance and how the corresponding effector is recognized. (D) **YrU1-mediated resistance.** In the proposed model, the effector binds to the WRKY domain, resulting in conformational changes and oligomerization of YrU1 proteins through the ANK and CC domains. (E) **BED-NLR-mediated resistance.** In this case, the BED domains, which substitute the CC domain in canonical NLR proteins, might act as decoys for pathogen effector targets. Consequently, on effector recognition by the BED domain, conformational changes and oligomerization could lead to activation. (F) **NLR-mediated resistance.** Canonical NLRs might activate via direct, indirect (guard/decoy), and integrated decoy recognition models. Here, Sr35-mediated resistance is shown, where the Sr35 NLR protein directly recognizes the AvrSr35 protein through the LRR domains, activating disease resistance. (G) **Pm4b-mediated resistance.** In the absence of the AvrPm4, Pm4b_V1 and Pm4b_V2 form an endoplasmic reticulum (ER)-associated heterocomplex interacting via C2 domains. On AvrPm4 recognition by the kinase domain, the heterocomplex might undergo conformation changes, leading to activation of the kinase activity and disease resistance.

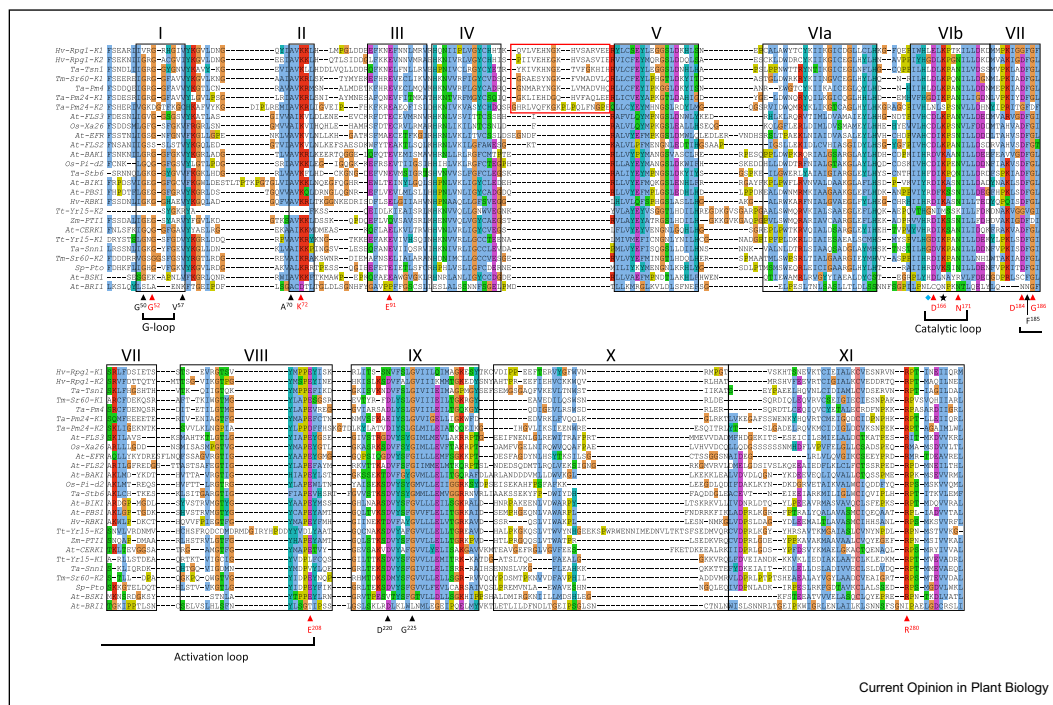
range of biological functions [49], including pathogen resistance. The wheat *Stb6* gene [50] confers race-specific resistance against the fungus *Zymoseptoria tritici* by detecting the presence of a matching apoplastic effector [51,52] in a gene-for-gene manner without hypersensitive response [52] (Figure 1). This differs from the previous WAKs conferring pathogen resistance, like the maize *Htm1* which confers partial, QR to northern corn leaf blight [53,54]. In an inverse gene-for-gene interaction, the wheat WAK-encoding *Snn1* gene [55] is hijacked by the necrotrophic toxin SnToxA, which is directly recognized by the Snn1 protein [56] (Figure 1). Both SnToxA as well as AvrStb6 recognized by Stb6 are proteins, suggesting that WAK protein receptors can

recognize proteinaceous effectors in addition to oligogalacturonide ligands [57]. Finally, the recent cloning of *rcs5* revealed two tightly linked genes, *Sbs1* and *Sbs2*, encoding two WAK proteins that act as susceptibility targets of the barley hemibiotrophic fungal pathogen *Bipolaris sorokiniana* [58]. Genes encoding WAKs are abundant in cereal genomes, and there is a need for more studies on their contribution to race-specific as well as quantitative disease resistance in cereals.

Novel types of race-specific resistance genes

The development of nonbiased gene isolation strategies in cereals such as MutChromSeq [59] has resulted in

Figure 2



Predicted TKP and Pm4b kinase catalytic domains. A multiple amino acid sequence alignment of 23 protein kinase catalytic domains mostly involved in cereal disease resistance was used to infer the kinase domain architecture of TKP and Pm4b proteins. The alignment was performed using Clustal Omega program and displayed in the ClustalX color mode in JaviView 2.8. Conserved domains I through XI are highlighted in boxes. Red arrowheads mark invariant residues (G⁵², K⁷², E⁹¹, D¹⁶⁶, N¹⁷¹, D¹⁸⁴, G¹⁸⁶, E²⁰⁸, R²⁸⁰), which are numbered with upper case numbers corresponding to their position in the α form of the cAMP-dependent protein kinase catalytic unit (cAPK). Likewise, black arrowheads indicate the most invariant residues (G⁵⁰, V⁵⁷, F¹⁸⁵, D²²⁰, G²²⁵). Based on the presence of an L residue at position R¹⁶⁵ of cAPK in subdomain VI, TKP and Pm4 Kin domains were classified as non-RD kinases. Moreover, conserved residues in subdomains VI (D¹⁶⁶ > N¹⁷¹) and VIII (GT/SXXY/FXAPE) indicate that the TKP and Pm4 encode kinase proteins with serine/threonine protein specificity. Intriguingly, TKPs (except Yr15) and Pm4 kinase domains share a conserved sequence (highlighted with a red box in the top part of the alignment) between domains IV and V absent in the rest of kinase-containing PRRs. Labels: red and black arrowheads, key invariant and nearly invariant residues in the protein kinase catalytic domains, respectively. Light blue diamond points to the RD or non-RD kinase determination site. Species abbreviation: At, *Arabidopsis thaliana*; Hv, *Hordeum vulgare*; Os, *Oryza sativa*; Sp, *Solanum lycopersicum*; Ta, *Triticum aestivum*; Tt, *Triticum turgidum*; Tm, *Triticum monococcum*; Zm, *Zea mays*. UniProt accession numbers are as follows: BAK1 (Q94F62), BIK1 (Q48814), BRI1 (Q22476), BSK1 (Q944A7), CERK1 (A8R7E6), EFR (C0LGT6), FLS2 (Q9FL28), FLS3 (A0A3Q7FWR4), PBS1 (Q9FE20), Pi-d2 (Q0DC23), Pm24 (A0A6B9QRM0), Pto (P93215), PTI1 (B4FZN9), RBK1 (I2FLM1), Rpg1 (Q8L3P8), Snn1 (W5AB81), Sr60 (A0A5C1K2R9), Stb6 (A0A1D5VHV3), Tsn1 (D7PDA0), Yr15 (A0A2Z5GZM3), Za26 (Q2EZ14). Pm4b has been submitted under GenBank accession numbers MT783929 and MT783930. Note that each protein name is preceded by the species abbreviation. Finally, in the case of TKP proteins, K1 and K2 state for the first and second kinase domain, respectively.

the molecular identification of immune receptors with unique, previously unknown, domain architectures. The *Lr14a* gene conferring resistance to the wheat leaf rust pathogen was found to encode a chimeric protein with an N-terminal ankyrin repeat domain and a C-terminal transmembrane domain [60] with overall protein sequence homology to the *Arabidopsis* ACD6 protein. *Lr14a* also shows similarity to the human transient receptor potential ankyrin channels that are Ca²⁺-permeable nonselective cation channels (Figure 1). The *Pm4* gene encodes a chimeric protein of a serine–threonine kinase and multiple C2-domains and transmembrane regions [61]. Functional analysis of Pm4 revealed that two protein variants resulting from constitutive alternative splicing are needed for resistance and that the

two encoded protein variants interact biochemically forming an endoplasmic reticulum–associated complex (Figure 1). Pm4 shows homology to *Arabidopsis* proteins located in plasmodesmata, suggesting the unidentified AvrPm4 effector could be recognized at the plasmodesmata. Both *Lr14a* and Pm4 will undoubtedly reveal novel molecular mechanisms for achieving race-specific plant immunity.

Future research directions and open questions

In contrast to the well-studied interactions of NLRs with effectors, the molecular analysis of non–NLR-based race-specific resistance is at an early stage. It will be important to isolate the corresponding pathogen avirulence genes,

which will also allow to identify host targets and to understand their relationship with resistance genes. *AvrStb6* is the only known avirulence gene corresponding to a non-NLR protein [51,52]. It has the typical characteristics of a short, secreted protein with no homologies to known proteins, very similar to avirulence proteins recognized by NLRs. *Avr* gene identification might rapidly advance for stem rust and powdery mildew resistance genes where there has been progress in the identification of several avirulence genes recognized by NLR immune receptors (Table 1) [62–66]. Such work is essential to determine if non-NLR race-specific resistance genes, particularly the group encoding kinase domain proteins, function independently of NLR action or if they are guarders of nonpolymorphic and possibly redundant NLR proteins, similar to the *Pto*–*Prf* interaction in tomatoes where the *Pto* kinase is the target of the pathogen effector [47] (Figure 1).

It will also be important to study the resistance phenotypes of single non-NLR genes in defined, susceptible genetic backgrounds, either by backcrossing or by the development of transgenic lines. Furthermore, the relevance of the genetic background for gene function must be studied in detail: for example, the protein encoded by *Lr14a* confers a unique resistance phenotype, which depends on several modifier genes [67]. The further characterization of modifier genes will give insight into molecular mechanisms of gene function. Finally, all the novel types of resistance proteins must be explored for use in agriculture and for possible improvement by mutational changes. The future isolation of a large number of the genetically described resistance genes in cereals and their corresponding *Avr* genes will establish the whole interactome of R–Avr proteins and provide the basis for the development of effective and durable strategies to combat cereal diseases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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